

FORM PTO-1300 (Modified)
(REV 10-95)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

10648-0001-0 PCT

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/446109

INTERNATIONAL APPLICATION NO.
PCT/AU98/00490

INTERNATIONAL FILING DATE
25 JUNE 1998

PRIORITY DATE CLAIMED
25 JUNE 1997

TITLE OF INVENTION

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS AND G PROTEIN-COUPLED RECEPTORS

APPLICANT(S) FOR DO/EO/US

David FAIRLIE, et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☐ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Notice of Priority

PCT/IB/304

Written Opinion (2)

Request For Consideration of Documents Cited in International Search Report

Docket No.: 10648-0001-0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :
DAVID FAIRLIE ET AL : ATTN: APPLICATION DIVISION
SERIAL NO: NEW APPLICATION :
(BASED ON PCT/AU98/00490)
FILED: HERewith :
FOR: CYCLIC AGONISTS AND
ANTAGONISTS OF C5A
RECEPTORS AND G PROTEIN-
COUPLED RECEPTORS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE CLAIMS

Please delete Claims 24-32.

Please amend the claims as follows.

Claim 3, line 1, delete "or Claim 2".

Claim 4, line 1, replace "3" with --1--.

Claim 5, lines 1-2, replace "any one of Claims 1 to 4" with --Claim 1--.

Claim 7, lines 1-2, replace "any one of claims 1 to 4" with --Claim 1--.

Claim 8, lines 1-2, replace "any one of Claims 1 to 4" with --Claim 1--.

Claim 9, lines 1-2, replace "any one of Claims 1 to 7" with --Claim 1--.

Claim 13, line 1, replace "any one of Claims 10 to 12" with --Claim 10--.

Claim 14, lines 1-2, replace "any one of Claims 3 and 10 to 13" with --Claim 13--.

Claim 17, line 1, delete "or Claim 16".

Claim 18, lines 1-2, replace "any one of Claims 15 to 17" with --Claim 15--.

Claim 20, line 2, replace "any one of Claims 1 to 19" with --Claim 1--.

Claim 21, line 4, replace "any one of Claims 1 to 19" with --Claim 1--.

REMARKS

Claims 1-23 are active in this application.

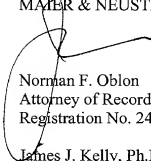
The claims have been amended to remove multiple dependencies. No new matter is believed to have been added to this application by these amendments.

Applicants submit that the present application is ready for examination on the merits.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAYER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

James J. Kelly, Ph.D.
Registration No. 41,504

Crystal Square Five - Fourth Floor
1755 Jefferson Davis Highway
Arlington, VA 22202
(703) 413-3000
Fax #: (703) 413-2220
NFO/JK

I:\atty\JK\106480001.PR

09/12/99
23 DEC 07

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS
AND G PROTEIN-COUPLED RECEPTORS

This invention relates to novel cyclic compounds which have the ability to modulate the activity of G protein-coupled receptors. The invention provides both agonists and antagonists. In preferred embodiments, the invention provides cyclic peptidic and cyclic or non-cyclic non-peptidic antagonists or agonists of C5a. The compounds of the invention are both potent and selective, and are useful in the treatment of a variety of inflammatory conditions.

BACKGROUND OF THE INVENTION

Activation of human complement, a system of plasma proteins involved in immunological defence against infection and injury, contributes significantly to the pathogenesis of numerous acute and chronic diseases. In particular, the complement protein C5a has been extensively investigated. For general reviews, see Whaley (1987), and Sim (1993). Table 1 provides a summary of known roles of C5a in disease.

During host defence, the complement system of plasma proteins initiates inflammatory and cellular immune responses to stimuli such as infectious organisms (bacteria, viruses, parasites), chemical or physical injury, radiation or neoplasia. Complement is activated through a complex cascade of interrelated proteolytic events that produce multiple bioactive peptides, some of which (eg. anaphylatoxins C3a and C5a) interact with cellular components to propagate inflammatory processes. Complement activation, either by the classical pathway, after antigen-antibody (Ag/Ab) binding, or by the antibody-independent alternate pathway, ends with a terminal sequence in which protein C5 is proteolytically cleaved by C5 convertase to C5a and C5b. The latter facilitates assembly of a "membrane attack complex" that punches holes

- 2 -

in membranes of target cells such as bacteria, leading to leakage, lysis and cell death. Steps in the cascade are tightly regulated to avoid stepwise amplification of proteolysis by sequentially formed proteases. If these
5 regulatory mechanisms become inefficient, protracted activation of complement can result, causing enhanced inflammatory responses as in autoimmune diseases.

Although the broad features of the complement system and its activation are known, mechanistic details
10 remain poorly understood. A principal and very potent mediator of inflammatory responses is the plasma glycoprotein C5a, which interacts with specific surface receptors (C5aR) on mast cells, neutrophils, monocytes, macrophages, non-myeloid cells, and vascular endothelial
15 cells (Gerard and Gerard, 1994). C5aR is a G protein-coupled receptor with seven transmembrane helices (Gerard and Gerard, 1991). This receptor is one of the rhodopsin superfamily of GTP-linked binding proteins, but differs from rhodopsin receptors in that the receptor and G protein
20 are linked prior to activation.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 80% of known cellular receptor types, and mediate signal
transduction across the cell membrane for a very wide range
25 of endogenous ligands. They participate in a diverse array of physiological and pathophysiological processes, including, but not limited to those associated with cardiovascular, central and peripheral nervous system, reproductive, metabolic, digestive, immunoinflammatory, and
30 growth disorders, as well as other cell-regulatory and proliferative disorders. Agents, both agonists and antagonists, which selectively modulate functions of G protein-coupled receptors have important therapeutic applications.

35 C5a is one of the most potent chemotactic agents known, and recruits neutrophils and macrophages to sites of injury, alters their morphology; induces degranulation;

- 3 -

increases calcium mobilisation, vascular permeability (oedema) and neutrophil adhesiveness; contracts smooth muscle; stimulates release of inflammatory mediators (including histamine, $\text{TNF-}\alpha$, IL-1, IL-6, IL-8, prostaglandins, leukotrienes) and lysosomal enzymes; promotes formation of oxygen radicals; and enhances antibody production (Gerard and Gerard, 1994). Overexpression or underregulation of C5a is implicated in the pathogenesis of immunoinflammatory conditions such as rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, lung injury and extracorporeal post-dialysis syndrome, and in a variety of other conditions, as summarised in Table 1.

Table 1
The Role of C5a in Disease

Condition/disease	C5a levels	C5ar expression	Details
allergy	++		allergen challenge leads to nasal symptoms and increased C5a levels
Alzheimer's disease	++	++	up-regulation of the receptor in reactive astrocytes, microglia and endothelial cells in the CNS, complement system activated by β -amyloid
ARDS/respiratory distress	++		
Behcet's disease	++		levels highest just prior to ocular attack
bronchial asthma	++		
capillary leak syndrome	++		

Condition/disease	C5a levels	C5aR expression	Details
chronic lung disease	++		Increased C5a levels in pulmonary effluent fluid from mechanically ventilated infants with chronic lung disease
Churg-Strauss			hypersensitivity of granulocytes to C5a
cystic fibrosis			generation of C5a/effects on PMNs
decompression stress	++		increased C5a levels during saturation diving
diabetes type I	++		C5a generated during onset; circulating monocytes in newly diagnosed type 1 diabetes patients are activated
Familial			lack of C5a inactivator
Mediterranean fever			
Guillain-Barre	++		CSF levels elevated
ischaemic disease			migration of monocytes into myocardium after reperfusion. Damage prevented with sCR1
states/ myocardial infarct			

Condition/disease	C5a levels	C5aR expression	Details
Kimura's disease			humoral factor up-regulates the response of PMNs to C5a
Multiple Sclerosis	++		increased expression of the receptor on foamy macrophages in acute and chronic MS and fibrous astrocytes in chronic MS
Meningitis			C5a induces experimental meningitis; PMN accumulation seen in the CSF
pancreatitis	++		
post-dialysis syndrome	++	-	C5a generated via complement activation by tubing material, C5aR levels decreased on PMNs & monocytes in chronic state
preeclampsia/ HELLP	++		C5a levels elevated at delivery
psoriasis	++		C5a levels high in scales
reperfusion injury	++		inhibited by C5 antibody
retinitis	++		C5a detected in vitreous humor

Condition/disease	C5a levels	C5aR expression	Details
Rheumatoid arthritis	++		elevated concentration of C5a found in synovial fluid (5-fold) and plasma (3-fold)
Severe congenital neutropenia	-		
transplant/graft rejection	++		monoclonal antibodies block the damage seen with xenogenic transplant; increased levels of C5a seen in the plasma and urine of patients with renal graft rejection

- 8 -

New agents which limit the pro-inflammatory actions of C5a have potential for inhibiting chronic inflammation, and its accompanying pain and tissue damage. For these reasons, molecules which prevent C5a binding to its receptors are useful for treating chronic inflammatory disorders driven by complement activation. Importantly, such compounds provide valuable new insights to mechanisms of complement-mediated immunity.

In another context, agonists of C5a receptors or other G protein-coupled receptors may also be found to have therapeutic properties in conditions either where the G protein-coupled receptor can be used as a recognition site for drug delivery, or where triggering of such receptors can be used to stimulate some aspect of the human immune system, for example in the treatment of cancers, viral or parasitic infections.

One approach to the development of agonists or antagonists of C5a is through receptor-based design, using knowledge of the three-dimensional structures of C5a, its receptor C5aR, and the interactions between them. The structure of the receptor is unknown. The solution structure of human C5a, a 74 amino acid peptide that is highly cationic and N-glycosylated with a 3 kDa carbohydrate at Asn64, has been determined and is essentially a 4-helix bundle. The C-terminal end (residues 65-74, C5a₆₅₋₇₄) was found to be unstructured (Zuiderweg et al, 1989) and this conformational flexibility in the C-terminus has made structure-function studies extremely difficult to interpret.

C5a has a highly ordered N-terminal core domain (residues 1-64; C5a₁₋₆₄), consisting of a compact antiparallel 4-helix bundle (residues 4-12, 18-26, 32-39, 46-63) connected by loops (13-17, 27-31, 40-45), and further stabilised by 3 disulphide bonds (C21-Cys47, Cys22-Cys54, Cys34-Cys55).

Although the structure of the C5a receptor, C5aR, is unknown, the C5a-binding subunit of human monocyte-

- 9 -

derived C5aR has been cloned and identified as a G protein-coupled receptor with transmembrane helices (Gerard and Gerard, 1991). Interactions between C5a and C5aR have been the subject of many investigations which, in summary, suggest that C5a binds via a two-site mechanism in which the N-terminal core domain of C5a is involved in receptor-recognition and binding, while the C-terminus is responsible for receptor activation. This mechanism is illustrated schematically in Figure 1. The C-terminal "effector" region alone possesses all the information necessary for signal transduction, and is thought to bind in the receptor's interhelical region (Siciliano *et al*, 1994; deMartino *et al*, 1995).

An N-terminal interhelical positively-charged region of C5a is responsible for receptor recognition and binding, and binds to a negatively-charged extracellular domain of C5aR (site 1), while the C-terminal "effector" region of C5a is thought to bind with the interhelical region of the receptor (site 2), and is responsible for receptor activation leading to signal transduction (Siciliano *et al*, 1994).

Numerous short peptide derivatives of the C-terminus of C5a have been found to be agonists of C5a (Kawai *et al*, 1991; Kawai *et al*, 1992; Kohl *et al*, 1993; Drapeau *et al*, 1993; Ember *et al*, 1992; Sanderson *et al*, 1994; Sanderson *et al*, 1995; Finch *et al*, 1997; Tempero *et al*, 1997; Konteatis *et al*, 1994; DeMartino *et al*, 1995). The structures of some of these agonists are shown in Table 2 below (compounds 1-6). High molecular weight polypeptide inhibitors of the action of C5a at its receptor, such as monoclonal antibodies to the C5a receptor, are also known (Morgan *et al*, 1992).

A small molecule, N-methylphenylalanine-lysine-proline-D-cyclohexylalanine-tryptophan-D-arginine (7, MeF-K-P-dCha-W-R), is a full antagonist of the C5a receptor, with no agonist activity when tested on isolated cellular membranes (Konteatis *et al*, 1994) or intact whole cells.

- 10 -

This hexapeptide was developed by modifications of the agonist NMe-F-K-P-dCha-L-r, in which the molecule was progressively substituted at leucine residues with substituents of increasing size (Cha, F, Nph and W). This

5 had the effect of reducing agonist activity. Receptor-binding assays, performed on isolated human neutrophil membranes, showed that the antagonist had only 0.04% relative affinity of C5a for the receptor (Konteatitis et al, 1994). A key feature of these reports is the definition of

10 the binding of **7** to the C5a receptor. These authors state that the C-terminal arginine is essential for receptor binding and antagonist activity. This is also the case in all the reports of agonist activity by small peptide analogues of the C-terminus of C5a. However, for the

15 antagonist **7**, the authors go further and state that
"the C-terminal carboxylate is an essential requirement for antagonist activity and receptor binding."

They proposed that the requirement of the

20 carboxylate is probably the result of its specific interaction with an arginine (Arg 206) in the receptor (De Martino et al, 1995). This idea was supported by a great reduction in receptor-affinity for an analogue of **7** in which the D-arginine ($\text{NH}_2\text{-CH}(\text{CO}_2\text{H})\text{-(CH}_2)_3\text{NHC}(:\text{NH})\text{NH}_2$) was

25 replaced by agmatine ($\text{NH}_2\text{-CH}_2\text{-(CH}_2)_3\text{NHC}(:\text{NH})\text{NH}_2$). In summary, De Martino et al claim that the D-arginine interacts via its guanidinium side chain with a negatively-charged amino acid side chain in the receptor. A second interaction between the negatively-charged C-terminal

30 carboxylate of **7** and a positively-charged side chain residue in the receptor is also thought to occur.

We have now determined the solution structure of this hexapeptide **7** and several analogues, and have surprisingly found that in fact a terminal carboxylate

35 group is not required for binding to C5aR or for antagonist activity, and that instead an unusual hitherto unrecognised structural feature, a turn conformation, is responsible for

- 11 -

C5a antagonist or agonist binding and activity. The hexapeptide and several new structurally related antagonists have been examined for both their receptor-binding affinities and antagonist activity, using intact
5 polymorphonuclear (PMN) cells. Our results show the hitherto unknown specific structural requirement for the binding of C5a antagonists or agonists to the C5a receptor, which we believe to be common to ligands for the G protein-coupled receptor family. Our establishment of this
10 specific structural requirement has enabled us to design and develop improved molecular probes of the complement system and of C5a-based drugs, and to design small molecules that target other G protein-coupled receptors, which are becoming increasingly recognised as important
15 drug targets due to their crucial roles in signal transduction (G protein-coupled Receptors, IBC Biomedical Library Series, 1996).

Thus our results have enabled us to design constrained structural templates which enable hydrophobic
20 groups to be assembled into a hydrophobic array for interaction with a G protein-coupled receptor, for example at Site 2 of the C5a receptor illustrated in Figure 1. Such templates or scaffolds, which may be cyclic or acyclic, have not heretofore been suggested for modulators
25 of the activity of C5a receptors or other G protein-coupled receptors.

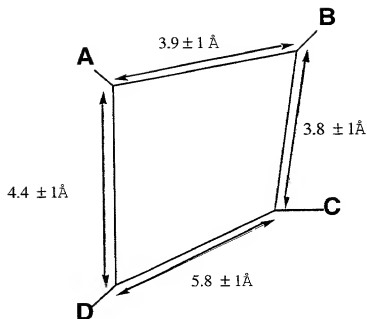
SUMMARY OF THE INVENTION

The invention provides cyclic and non-cyclic
30 modulators of the activity of G-protein-coupled receptors.

According to a first aspect, the invention provides a compound which is an antagonist, of a G protein-coupled receptor, which has no agonist activity, and which has a cyclic or constrained acyclic structure adapted to
35 provide a framework of approximate dimensions as follows:

- 12 -

Structure I

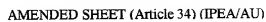


5

where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives, and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

where the critical amino acid side chains are designated by A, B, C and D, or are as defined below;

A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position, including, but not limited to the following side chains and other mimetics of arginine side chains:



- 14 -

where

X is NCN, NNO₂, CHNO₂ or NSO₂NH₂;

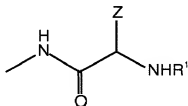
n is an integer from 1 to 4, and

R is H or an alkyl, aryl, CN, NH₂ or OH group .

5 B is any common or uncommon aromatic amino acid side chain which serves to position an aromatic side-chain group in this position, including but not limited to the indole, indole methyl, benzyl, phenyl, naphthyl, naphthyl methyl, cinnamyl group, or any other derivatives of these
10 aromatic groups;

C is any common or uncommon hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position, including, but not limited to D- or L-cyclohexyl alanine (Cha), leucine,
15 valine, isoleucine, phenylalanine, tryptophan, or methionin

D is any common or uncommon aromatic amino acid which serves to position an aromatic side-chain in this position, and has the structure:



where Z includes but is not limited to indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or any other derivatives of these aromatic groups, and

25 R¹ is H or any alkyl, aromatic, acyl or aromatic-acyl group including, but not limited to methyl, ethyl, propyl, butyl, -CO-CH₂CH₃, -CO-CH₃, -CO-CH₂CH₂CH₃, -CO-CH₂Ph, or -CO-Ph.

30 Preferably the G protein-coupled receptor is a C5a receptor.

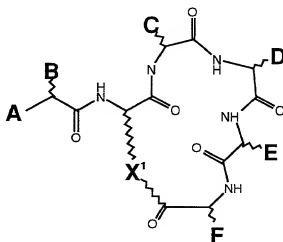
Other cyclic or constrained acyclic molecules, which may be peptidic or non-peptide in nature, can similarly be envisaged to support groups such as A, B, C

- 15 -

and D for interaction with a C5a receptor or other G protein-coupled receptor.

In one preferred embodiment, the compound has antagonist activity against C5aR, has no C5a agonist activity, and has the general formula:

Structure II



where A is H, alkyl, aryl, NH₂, NHalkyl, N(alkyl)₂, NHaryl or NHacyl; OH, Oalkyl, Oaryl.

B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid selected from phenylalanine, homophenylalanine, tryptophan, homotryptophan, tyrosine, and homotyrosine;

C is the side chain of a D-, L- or homo-amino acid selected from the group consisting of proline, alanine, leucine, valine, isoleucine, arginine, histidine, aspartate, glutamate, glutamine, asparagine, lysine, tyrosine, phenylalanine, cyclohexylalanine, norleucine, tryptophan, cysteine and methionine;

D is the side chain of a D- or L-amino acid selected from the group consisting of cyclohexylalanine, homocyclohexylalanine, leucine, norleucine, homoleucine, homonorleucine and tryptophan;

- 16 -

E is the side chain of a D- or L-amino acid selected from the group consisting of tryptophan and homotryptophan;

5 F is the side chain of a D- or L-amino acid selected from the group consisting of arginine, homoarginine, lysine and homolysine; and

X¹ is $-(CH_2)_nNH-$ or $(CH_2)_nS-$, where n is an integer of from 1 to 4, preferably 2 or 3, $-(CH_2)_2O-$, $-(CH_2)_3O-$, $-(CH_2)_3-$, $-(CH_2)_4-$, or $-CH_2COCH_2RNH-$, where R is the
10 side chain of any common or uncommon amino acid.

For the purposes of this specification, the term "alkyl" is to be taken to mean a straight, branched, or cyclic, substituted or unsubstituted alkyl chain of 1 to 6, preferably 1 to 4 carbons. Most preferably the alkyl group
15 is a methyl group. The term "acyl" is to be taken to mean a substituted or unsubstituted acyl of 1 to 6, preferably 1 to 4 carbon atoms. Most preferably the acyl group is acetyl. The term "aryl" is to be understood to mean a substituted or unsubstituted homocyclic or heterocyclic
20 aryl group, in which the ring preferably has 5 or 6 members.

A "common" amino acid is a L-amino acid selected from the group consisting of glycine, leucine, isoleucine, valine, alanine, phenylalanine, tyrosine, tryptophan,
25 aspartate, asparagine, glutamate, glutamine, cysteine, methionine, arginine, lysine, proline, serine, threonine and histidine.

An "uncommon" amino acid includes, but is not restricted to, D-amino acids, homo-amino acids, N-alkyl
30 amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), ortho-, meta- or para-aminobenzoic acid, ornithine, citrulline, norleucine, γ -glutamic acid, aminobutyric acid and α,α -disubstituted amino acids.

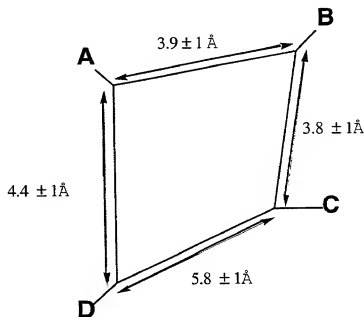
35 For the purposes of this specification it will be clearly understood that the word "comprising" means

- 17 -

"including but not limited to", and that the word "comprises" has a corresponding meaning.

According to a second aspect, of the invention provides a compound which is an agonist of G protein-coupled receptors, and which has structure III

Structure III



10

where the numerals refer to distances between C_{α} carbons of amino acids or their analogues or derivatives, and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

15

where B is a non-aromatic amino acid, and is preferably the D- or L-form of alanine, leucine, valine, norleucine, glutamic acid, aspartic acid, methionine, cysteine, isoleucine, serine or threonine,

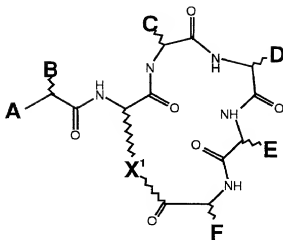
and A, C and D are as defined above.

20

Preferably the compound is of structure IV,

- 18 -

Structure IV



- 5 where E is any amino acid other than tryptophan and homotryptophan, for example D- or L- forms of alanine, leucine, valine, norleucine, phenylalanine, glutamic acid, aspartic acid, methionine, cysteine, isoleucine, serine, threonine, and F and X' are as defined in Structure II.
- 10 Preferably the compound is an agonist of C5a.

 According to a third aspect, the invention provides a composition, comprising a compound according to the invention together with a pharmaceutically-acceptable carrier or excipient.

- 15 The compositions of the invention may be formulated for oral or parenteral use, but oral formulations are preferred. It is expected that most if not all compounds of the invention will be stable in the presence of digestive enzymes. Such stability can readily
- 20 be tested by routine methods known to those skilled in the art.

- Suitable formulations for administration by any desired route may be prepared by standard methods, for example by reference to well-known textbooks such as
- 25 Remington; The Science and Practice of Pharmacy, Vol. II, 1995 (19th edition), A.R. Gennaro (ed), Mack Publishing Company, Easton, Pennsylvania, or Australian Prescription Products Guide, Vol. 1, 1995 (24th edition) J. Thomas (ed),

- 19 -

Australian Pharmaceutical Publishing Company Ltd, Victoria, Australia.

In a fourth aspect, the invention provides a method of treatment of a pathological condition mediated by a G protein-coupled receptor, comprising the step of administering an effective amount of a compound of the invention to a mammal in need of such treatment.

Preferably the condition mediated by a G protein-coupled receptor is a condition mediated by a C5a receptor, and more preferably involves overexpression or underregulation of C5a. Such conditions include but are not limited to rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, lung injury and extracorporeal post-dialysis syndrome.

While the invention is not in any way restricted to the treatment of any particular animal or species, it is particularly contemplated that the compounds of the invention will be useful in medical treatment of humans, and will also be useful in veterinary treatment, particularly of companion animals such as cats and dogs, livestock such as cattle, horses and sheep, and zoo animals, including large bovids, felids, ungulates and canids.

The compounds may be administered at any suitable dose and by any suitable route. Oral administration is preferred because of its greater convenience and acceptability. The effective dose will depend on the nature of the condition to be treated, and the age, weight, and underlying state of health of the individual treatment. This will be at the discretion of the attending physician or veterinarian. Suitable dosage levels may readily be determined by trial and error experimentation, using methods which are well known in the art.

- 20 -

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a diagrammatic representation of the two-site model for binding of C5a to its G protein-coupled receptor, C5aR. The black rods represent α -helical regions, and the open cylinders represent the transmembrane helices. Sites 1 and 2 are indicated on the figure.

Figure 2 shows stacked plots of ^1H -NMR spectra, showing time-dependent decay of amide NH resonances for Trp (8.10 ppm) and D-Cha (7.90 ppm) residues of **7** in d_6 -DMSO containing D_2O after 10 minutes (bottom plot) and then 25, 40, 55, 70, 130, 190, 250, 385 and 520 minutes.

Figure 3 shows backbone C, N, O atoms of twenty lowest energy minimized NMR structures of **7** in d_6 -DMSO at 24°C).

Figure 4 shows a schematic representation of H-bonding in the structure of **7** from proton NMR spectra in d_6 -DMSO.

Figure 5a shows (a) receptor binding, as indicated by inhibition of binding of ^{125}I -C5a to human PMNs by **7** (●); **8** (Δ); **9** (▲); **12** (○).

Figure 5b shows C5a antagonist potency as inhibition of myeloperoxidase (MPO) release from human PMNs by : **7** (■, n= 9) and **12** (▲, n=4). Figure 5c shows C5aR binding and antagonist potencies of **7**, **15** and **17**.

A-C show the effect of increasing concentrations (top to bottom) of C5a antagonists inhibiting myeloperoxidase release in human PMNs (n=3 in A-C).

A: **7** at 0, 0.1, 0.3, 1.0 μM (top to bottom)

B: **15** at 0, 0.1, 0.03, 0.1 μM (top to bottom)

C: **17** at 0, 0.01, 0.03, 0.1 μM (top to bottom)

D: Comparative affinities for PMN C5qR receptor. Inhibition of binding of ^{125}I -C5a to human PMNs by **7** (top), **15** (middle), **17** (bottom). All data are means \pm SEM.

Figure 6 shows receptor binding of cyclic C5a antagonists, as shown by inhibition of binding of ^{125}I -C5a to human PMNs (n=5).

- 21 -

Figure 7 shows superimposed structures of **7** (light, NMR structure) and **12** (dark, computer modelled structure). Phe and Trp side chains are omitted from **12** for clarity.

Figure 8 shows inhibition of C5a-induced neutropenia in Wistar rats by the cyclic antagonist F-[OPdChaWR] given i.v. at 1mg/kg. Results shown from n=3 in each group, *P<0.05 compared to C5a-treated group only. Results are expressed as mean \pm SEM.

Figure 9 shows inhibition of LPS-induced neutropenia and changes in haematocrit induced by the cyclic antagonist F-[OPdChaWR] (0.03-10mg/kg, i.v., 10 min prior to lipopolysaccharide [LPS]) in Wistar rats. Abscissa: time after LPS (1 mg/kg i.v. injection). Ordinate: per cent change in haematocrit (A) value or level of circulating polymorphonuclear (PMN) leukocytes (B) compared to time zero.

Figure 10 shows inhibition of carrageenan-induced (Wistar) rat paw oedema by cyclic antagonist (3D35) AcF-[OPdChaWR] (1 mg/kg single dose i.p. given 30 min prior to carrageenan). Results shown from 4 rats /group, mean \pm SEM. Ordinate: percent change in paw volume. Abscissa: time (mins) after carrageenan injection

25 DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described by way of reference only to the following general methods and experimental examples, and to the figures. Abbreviations used herein are as follows:

- | | | |
|----|-------|---|
| 30 | BOP | benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate |
| | D-Cha | D-cyclohexylamine |
| | DIPEA | diisopropylethylamine |
| | DMF | dimethylformamide |
| 35 | DMSO | dimethylsulphoxide |
| | HBTU | O-benzotriazole N',N',N',N'-tetramethyluronium hexafluorophosphate; |

- 22 -

LPS lipopolysaccharide
PMN polymorphonuclear granulocyte
RMSD root mean square deviation
RP-HPLC reverse phase-high performance liquid
5 chromatography
TFA trifluoroacetic acid;

Throughout the specification conventional single-letter and three-letter codes are used to represent amino
10 acids.

General Methods

Protected amino acids and resins were obtained from Novabiochem. TFA, DIPEA and DMF (peptide synthesis
15 grade) were purchased from Auspep. All other materials were reagent grade unless otherwise stated. Preparative scale reverse-phase HPLC separations were performed on a Vydac C18 reverse-phase column (2.2 x 25 cm), and analytical reverse-phase HPLC separations were performed on a Waters
20 Delta-Pak PrepPak C18 reverse-phase column (0.8 x 10 cm), using gradient mixtures of solvent A = water/0.1% TFA and solvent B = water 10%/acetonitrile 90%, 0.09% TFA. The molecular weight of the peptides was determined by electrospray mass spectrometry recorded on a triple
25 quadrupole mass spectrometer (PE SCIEX API III), as described elsewhere (Haviland et al, 1995). ¹H-NMR spectra were recorded on either a Bruker ARX 500 MHz or a Varian Unity 400 spectrometer. Proton assignments were determined by 2D NMR experiments (DFCOSY, TOCSY, NOESY).

30 Non-peptidic compounds were synthesized using conventional organic chemical methods. Compounds were analysed by ¹H-NMR spectroscopy and by mass spectrometry.

Peptide synthesis

35 Some representative peptide syntheses are now given. Linear peptide sequences were assembled by manual step-wise solid-phase peptide synthesis with HBTU

- 23 -

activation and DIEA *in situ* neutralisation. Boc chemistry was employed for temporary N α -protection of amino acids with two 1 min treatments with TFA for Boc group removal. The peptides were fully deprotected and cleaved by

5 treatment with liquid HF (10 ml; p-cresol (1 ml); -5°C; 1-2 hr). Analytical HPLC (gradient; 0% B to 50% B over 40 min): 7, Rt = 32.0 min, [M+H]⁺(calc.) = 900.5, [M+H]⁺(exper.) = 900.7; 8, Rt = 32.2 min, [M+H]⁺(calc.) = 899.6, [M+H]⁺(exper.) = 899.7;

10 9, Rt = 30.0 min, [M+H]⁺(calc.) = 900.5, [M+H]⁺(exper.) = 900.7; 10, Rt = 23.8 min, [M+H]⁺(calc.) = 860.5, [M+H]⁺(exper.) = 860.5.

Structures for the peptides are shown in Table 4 below.

- 24 -

a) *Synthesis of cycle 11*

This is a general method used for the synthesis of a wide range of cyclic antagonists covered by this patent. For example, in the case of cycle 11, its linear precursor peptide was synthesised by Fmoc chemistry using HBTU/DIEA activation on an Fmoc-D-Arg(Mtr)-Wang resin. Fmoc group removal was effected using two 1 min treatments with 50% piperidine/DMF. Cleavage and deprotection using 95% TFA/2.5% TIPS/2.5% H₂O gave the Mtr-protected peptide, which was purified by RP-HPLC. Cyclization of the protected, purified peptide using 3eq BOP and 10eq DIEA at a 1 mM concentration in DMF stirring for 15 hr gave the cyclised product, which was fully deprotected using 1M TMSBr in TFA. A final RP-HPLC purification gave the desired peptide in yields of 50% for the cyclisation. Rt = 37.7 min, [M+H]⁺(calc.) = 910.5, [M+H]⁺(exper.) = 910.7.

b) *Synthesis of cycle 12*

Cyclization of the cleaved and fully deprotected peptide was achieved by stirring a 1mM solution in DMF with 3eq BOP and 10 eq pyridine as base for 15hr. A final RP-HPLC purification gave the desired peptide in yields of 22% for the cyclization. Rt = 37.3 min, [M+H]⁺(calc.) = 896.5, [M+H]⁺(exper.) = 896.5.

NMR Structure Determination

¹H-NMR spectra were recorded for compound 7 (3 mg in 750 µl d₆-DMSO, δ 2.50) referenced to solvent on a Varian Unity 400 spectrometer at 24°C. Two dimensional ¹H-NMR NOESY (relaxation delay 2.0s, mix time 50-300 ms), DFQ- COSY and TOCSY (mixing time 75ms) experiments were acquired and recorded in phase sensitive mode. Acquisition times = 0.186 s, spectral width = 5500Hz, number of complex points (t₁ dimension) = 1024 for all experiments. Data was zero-filled and Fourier transformed to 1024 real points in both dimensions.

- 25 -

NMR data was processed using TRIAD software (Tripos Assoc.) on a Silicon Graphics Indy work station. 2D NOE cross peaks were integrated and characterised into strong (1.8-2.5Å), medium (2.3-3.5Å) and weak (3.3-5.0Å).

5 Preliminary three-dimensional structures were calculated from upper and lower distance limit files using Diana 2.8 (69 distance constraints, including 27 for adjacent residues and 6 further away) with the redundant dihedral angle constraints (REDAC) strategy. Upper and lower

10 distance constraints were accurately calculated using MARDIGRAS. At this stage the peptide was examined for possible hydrogen bonds, and these were added as distance constraints. The 50 lowest energy Diana structures were subjected to restrained molecular dynamics (RMD) and energy

15 minimisation (REM). Initially, REM consisted of a 50 step steepest descent followed by 100 step conjugate gradient minimisation. RMD was performed by simulated heating of the structures to 300K for 1ps, followed by 500K for 1ps. The temperature was gradually lowered to 300K over 2ps and

20 finally for 2ps at 200K. REM was performed again with a 50 step steepest descent, 200 step conjugate gradient followed by a 300 step Powell minimisation. The final structures were examined to obtain a mean pairwise rms difference over the backbone heavy atoms (N, C α and C). Twenty of the 50

25 structures had a mean rmsd < 0.5 Å for all backbone atoms (O, N, C).

Molecular Modelling

A model of cycle 12, shown in Figure 7, was

30 created from the NMR structure of 7 by deleting all NMR constraints, fusing the ornithine side chain amine to the C-terminal carboxylate of d-Arg to form an amide, and minimising using Powell forcefield (1000 iterations). The modelled structure was then superimposed on the NMR

35 structure with an rmsd 0.224Å.

- 26 -

Receptor-Binding Assay

Assays were performed with fresh human PMNs, isolated as previously described (Sanderson et al, 1995), using a buffer of 50 mM HEPES, 1 mM CaCl_2 , 5 mM MgCl_2 , 0.5% bovine serum albumin, 0.1% bacitracin and 100 μM phenylmethylsulfonyl fluoride (PMSF). In assays performed at 4°C, buffer, unlabelled human recombinant C5a (Sigma) or peptide, Hunter/Bolton labelled ^{125}I -C5a (~ 20 pM) (New England Nuclear, MA) and PMNs (0.2×10^6) were added sequentially to a Millipore Multiscreen assay plate (HV 0.45) having a final volume of 200 μL /well. After incubation for 60 min at 4°C, the samples were filtered and the plate washed once with buffer. Filters were dried, punched and counted in an LKB gamma counter. Non-specific binding was assessed by the inclusion of 1 mM peptide or 100 nM C5a which typically resulted in 10-15% total binding.

Data was analysed using non-linear regression and statistics with Dunnett post test.

Myeloperoxidase Release

Cells were isolated as previously described (Sanderson et al, 1995) and incubated with cytochalasin B (5 $\mu\text{g}/\text{mL}$, 15 min, 37°C). Hank's Balanced Salt solution containing 0.15% gelatin and peptide was added on to a 96 well plate (total volume 100 μL /well), followed by 25 μL cells ($4 \times 10^6/\text{mL}$). To assess the capacity of each peptide to antagonise C5a, cells were incubated for 5 min at 37°C with each peptide, followed by addition of C5a (100 nM) and further incubation for 5 min. Then 50 μL of sodium phosphate (0.1M, pH 6.8) was added to each well, the plate was cooled to room temperature, and 25 μL of a fresh mixture of equal volumes of dimethoxybenzidine (5.7 mg/mL) and H_2O_2 (0.51%) was added to each well. The reaction was stopped at 10 min by addition of 2% sodium azide. Absorbances were measured at 450 nm in a Bioscan 450 plate

- 27 -

reader, corrected for control values (no peptide), and analysed by non-linear regression.

In Vivo Assays of Anti-Inflammatory Activity

5 The following well-known *in vivo* assay systems may be used to assess the anti-inflammatory activity of compounds of the invention. All assay data are analysed using non-linear regression analysis and Student's t-test, analysis of variance, with $p < 0.05$ as the threshold level of
10 significance.

(a) Carrageenan Paw Oedema

 Anaesthetised (i.p. ketamine & xylazine) Wistar rats (150-200g) or mice were injected with sterilised air (20ml day 1, 10ml day 4) into the subcutaneous tissue of the back. The cavity can be used after 6 days, whereupon carrageenan (2ml, 1% w/w in 0.9% saline) was injected into the air pouch and exudate was collected after 10 hr. Test compounds are administered daily after Day 6 and their
15 anti-inflammatory effects assayed by differential counting of cells in the air-pouch exudate. Animals were killed at appropriate times after injection and 2ml 0.9% saline was used to lavage the cavity, lavage fluids were transferred to heparinised tube and cells were counted with a
20 haemocytometer and Diff-Quik stained cytocentrifuged preparation.

 Alternatively, a routine carrageenan paw oedema was developed in Wistar rats by administering a pedal injection of carrageenan to elicit oedema which is visible
30 in 2h and maximised in 4h. Test compounds are given 40 min before inflammagen and evaluated by microcaliper measurements of paws after 2 & 4 hr. See Fairlie, D.P. et al (1987). Also see Walker and Whitehouse (1978).

(b) Adjuvant Arthritis.

 Adjuvant arthritis was induced in rats (3 strains) either microbially (injection of heat-killed

- 28 -

- Mycobacterium tuberculosis*) or chemically (with avridine) by inoculation with the arthritogenic adjuvant co-administered with oily vehicles (Freund's adjuvants) in the tail base. (See Whitehouse, M. W., Handbook of Animal Models for the Rheumatic Diseases, Eds. Greenwald, R. A.; Diamond, H. S.; Vol. 1, pp. 3-16, CRC Press) .

- Within 13 days the adjuvant arthritis is manifested by local inflammation and ulceration in the tail, gross swelling of all four paws, inflammatory lesions in paws and ears, weight loss and fever. These symptoms, which are similar to those of inflammatory disease in humans (Winter and Nuss, 1966), can be alleviated by agents such as indomethacin or cyclosporin which also show beneficial effects in man (eg. Ward and Cloud, 1966).
- Without drug treatment at Day 14, arthritic rats had hypertrophy of the paws, reduced albumin but raised acute phase reaction proteins in serum, and depressed hepatic metabolism of xenobiotics as indicated by prolonged barbiturate-induced sleeping times.

- To assess activity, compounds were administered for 4 days orally ($\leq 10\text{mg/kg/day}$) or i.p. from Days 10-13 following inoculation with arthritogen (Day 0). The inflammation was either not visible or very significantly reduced in rear or front paws as assessed by microcaliper measurements of paw thickness and tail volume, as well as by gross inspection of inflammatory lesions. Animals are sacrificed by cervical dislocation on Day 18 unless arthritis signs are absent, whereupon duration of observations is continued with special permission from the Ethics committees. Experiments are staggered to maximise throughput and allow early comparisons between compounds. This routine assay is well-accepted as identifying anti-inflammatory agents for use in humans.

- 29 -

Example 1 Structure-Activity Relationship of C5a
Agonists

We have focussed on the C-terminal residues of C5a, in order to explore structure-activity relationships in the search for peptide sequences with potent agonist activity. Many of these peptides are full agonists relative to C5a, but have markedly lower potency (Sanderson et al, 1994, 1995; Finch et al, 1997). Our initial structure-activity investigations have been particularly informative. Mutating the decapeptide C-terminus of C5a (1, C5a₆₅₋₇₄, ISHKDMQLGR) twice with I₆₅Y and H₆₇F (eg. 2) led to enhancement of agonist potency by about 2 orders of magnitude. These results are summarised in Table 2. Analyses of Ramachandran plots and 2D NMR spectra for compound 2 suggested that certain structural features, namely a twisted "helix-like" backbone conformation for residues 65-69 and a β -turn for residues 71-74, might be responsible for activity. These preliminary results provided some insight to structural requirements for tight binding to a C5a receptor.

Table 2
Pharmacological Activity of C5a Agonist Analogues*

Peptide No.	Peptide	Fetal Artery EC ₅₀ (μM)	PMN Enzyme Release EC ₅₀ (μM)	Binding Affinity IC ₅₀ (μM)
1	C5a ₆₅₋₇₄ (ISHKDMQLGR)	>1000	>1000	>1000
2	YSEKDMQLGR	9.6	92	1.3
3	YSEKDMPLaR	0.5	72	3.7
4	YSEKPMPLaR	0.2	4.1	6.0
5	C5a ₃₇₋₄₆ -ahxYSEKPMPLaR	0.06	5.9	0.7
6	C5a ₁₂₋₂₀ -ahxYSEKPMPLaR	0.08	0.7	0.07
	C5a	0.02	0.03	0.0006

* Finch et al, 1997

- 31 -

Compounds 4, 5 and 6 in Table 2 are the highest affinity small C5a agonists so far known, with up to 25% C5a potency in human fetal artery, 5% C5a potency in human PMN enzyme release assays and 1% C5a affinity for PMN C5aR (Finch et al., 1997). For the PMN receptor, these compounds have up to 100-fold higher apparent affinity than any small molecule previously described in the literature.

The "high" affinities (70nM-6 μ M) of these agonist analogues for C5aR in intact PMN cells have enabled us to identify a common topographical feature in peptide agonists that correlates with expression of spasmogenic activities and enzyme-release assays in human PMNs. This preferred backbone conformation is a type II β -turn.

The small size of these agonist peptides makes them amenable to synthetic modification to optimise their affinities, activities, and bioavailabilities, and hence useful as mechanistic probes of receptor activation.

Example 2 NMR Structure of C5a Antagonist

We used two dimensional nuclear magnetic resonance spectroscopy to determine the three dimensional structure of 7 and found that while there is no discernible structure in water, there is evidence of a stable gamma-turn structure in dimethylsulfoxide.

The 1D ^1H -NMR spectrum of peptide 7 in d_6 -DMSO at 24°C shows 4 distinct resonances for amide-NH protons, as summarized in Table 3. To establish their possible involvement in intramolecular hydrogen bonds, a deuterium exchange experiment was performed by adding a 10-fold excess of D_2O to the solution. Two of the amide-NH doublets disappeared immediately, along with resonances attributable to the N-terminal methylamine protons. However, the other two amide NH resonances, as well as a broad resonance at approximately 8.05 ppm, persisted for up to 6.5 hours (Figure 2). These three slowly-exchanging protons are assigned to the amide NHs of Trp and d-Cha and the side chain amine of Lys, the slow exchange behaviour

- 32 -

being characteristic of hydrogen-bonding. The amine assignment was established from the TOCSY spectrum where cross peaks were observed between the protonated amine and the ϵ , δ and γ CH_2 protons. A temperature dependence study

5 (20-60°C) of the amide-NH chemical shifts ($\Delta\delta/T = 2.5$ ppb/deg, dCha-NH; 6 ppb/deg, Trp-NH; 6.5 ppb, Lys-NH; 8.7ppb, Arg-NH) unambiguously confirmed the involvement of the dCha-NH only in intramolecular hydrogen bonding.

Table 3
¹H-NMR Assignments^a for 7 in d₅-DMSO

Residue	^b H _N	H _α	H _β	H _γ	Others
MePhe	-	4.06	3.09, 3.06	-	c 7.17, 7.29; d ₂ 46; f ₈ 98
Lys	8.83	4.54	1.74, 1.55	1.32	e ₁ 51; f ₂ 74, g ₇ 76 (NH ₂)
Pro	-	4.30	2.084, 1.74	1.88, 1.78	e ₃ 61, f ₃ 48
d-Cha	7.91	4.35	1.19, 1.06	0.76	e ₁ 43, 1.08; f ₁ 61, 1.58; 0.73
Trp	8.01	4.65	3.11, 2.94	-	c ₆ 97, 7.06, 7.13, 7.32, 7.65; g ₁₀ 80
d-Arg	8.44	4.20	1.73, 1.58	1.42	e ₃ 08; g ₇ 60

a Referenced to residual d₅-DMSO at 2.50 ppm.

b Amide NHs, ³J_{NH-CAH} values (Hz): 7.91 (Lys), 7.77 (d-Arg), 8.34 (Trp), 8.53 (d-Cha).

c Aromatics

d N-Me.

e Hδ.

f Hε

g NH/NH₂ amine.

- 34 -

A series of 2D ^1H -NMR spectra were measured for **7** at 24°C in d_6 -DMSO to determine the three-dimensional structure. TOCSY and DFQ-COSY experiments were used to identify residue types, while sequential assignments were made from analysis of NOESY data. From a series of 100 structures generated from NOESY data, fifty of the lowest energy structures were subjected to restrained molecular dynamics (200K-500K) and energy minimised. A set of 20 calculated structures with a root mean square deviation (rmsd) < 0.5 Å (backbone atoms) are superimposed in Figure 3, and clearly depict a turn conformation.

In combination, the NMR constraint data, $^3J_{\text{NH-COH}}$ values, deuterium exchange and temperature dependence data establish an unusual turn structure for hexapeptide **7** which is constrained by up to three hydrogen bonds, as shown in Figure 4. The evidence is very strong for one intramolecular hydrogen bond from dCha-NH...OC-Lys (2.72 Å, N-H...O angle 157°, C=O...H angle 84°), forming a 7-membered ring that defines an inverse γ -turn. The dChaNH-O-TrpNH angle is 56.4°. The deuterium exchange data and NMR constraint data together point to a second intramolecular hydrogen bond Trp-NH...OC-Lys (3.31 Å, N-H...O angle 159°, CO...H angle 137.3°) forming a 10-membered ring characteristic of a β -turn. The ϕ and ψ angles ($\phi_2 = -58.4^\circ$, $\psi_2 = 62.0^\circ$; $\phi = 96.6^\circ$, $\psi_3 = 16.6^\circ$) most closely match a type II β -turn (Bandeekar, 1993; Hutchinson and Thornton, 1994) which is distorted by the presence of the γ -turn wholly within the β -turn.

To our knowledge this is the first example of an intramolecular hydrogen bond between residues within a β -turn, although there are many examples of hydrogen bonds between a residue within the "10 membered ring" of a β -turn and a residue outside of it (Bandeekar, 1993). A third hydrogen bond (2.76 Å, N-H...O angle 160.3°), between the side-chain amine of Lys and the C-terminal carboxylate, is suggested by the NMR constraint data, by slow NH/ND exchange and by detection of a weak NOE between Lys-

- 35 -

NH...Trp- α CH₂. This may further constrain the molecule into the observed turn conformation. Such ion-pairing is common in dipolar aprotic solvents such as dimethylsulphoxide and may also be relevant in a

5 hydrophobic protein environment.

NMR solution structures have also been determined for several of the cyclic antagonists described in the following examples, and show that in each case the type II β -turn is preserved and stabilized by the cyclic structure.

10 The constraining β and γ turns proposed in the linear peptide **7** have parallels in cyclic peptides. We have previously detected overlapping β and γ turns in a cyclic octapeptide from ascidiacyclamide (Abbenante *et al*, 1996). Combinations of a β - and γ -turn have also been
15 found in the backbones of cyclic penta- and hexapeptides, particularly those containing alternating D- and L-amino acids (Marraud and Aubry 1996; Fairlie *et al*, 1995; Kessler *et al*, 1995; Stradley *et al*, 1990). For example a type II β -turn and an inverse γ -turn have been identified in cyclic
20 antagonists c-(D- Glu-Ala-D-allo-Ile-Leu-D-Trp) (Ihara *et al*, 1991; Coles *et al*, 1993; Ihara *et al*, 1992; Bean *et al*, 1994) and c-(D-Asp-Pro-D-Val-Leu-D-Trp) (Bean *et al*, 1994) for endothelin receptors, and in members of the rhodopsin family of G protein-coupled receptors with seven
25 transmembrane domains (X.-M.Cheng *et al*, 1994). In the latter case, as in **7**, an inverse γ -turn forms between residues (Asp-CO....Val-NH, Lys-CO....dCha-NH) that flank the proline.

30 Example 3 Structure-Activity Relationships In Vitro

We also examined the receptor-binding and antagonist activity of the hexapeptide **7** for comparison with our new compounds. The previous report by Konteatis *et al* (1994) concerned the ability of **7** to compete with C5a
35 binding to receptors on isolated PMN membranes (IC₅₀ 70 nM), which is not necessarily physiologically relevant. We examined competition between **7** and C5a using intact PMN

- 36 -

cells, and found that, under these conditions, **7** binds with much lower receptor affinity of IC_{50} 1.8 μ M. We confirmed that **7** is a full antagonist with no agonist properties. These results are summarized in Figure 5a and Table 4. The relative affinity (ratio) of **7** for the C5aR in intact PMNs in our assays was similar to that previously reported for isolated PMN membranes.

We have also found that **7** shows antagonist activity against both C5a (Figure 5b) and a C-terminal agonist decapeptide analogue **4** (YSFKPMPLaR) (Finch et al, 1997) of the C-terminus C5a₆₅₋₇₄, suggesting that it acts on site 2 of the receptor. Compounds **7** and **4** have similar μ M affinity for the receptor C5aR on intact polymorphonuclear leukocytes, as shown in Table 4.

A new discovery from the data in Table 4 is the linear correlation between the log of binding affinities and the log of antagonist potencies for these Site 2 antagonists (compounds **7-12**, Table 4). The importance of this linear relationship is that since receptor affinity and antagonist activity are directly proportional, the experimentally simpler approach of measuring receptor binding may be used to estimate the antagonist activity for such small compounds, provided that there is no evidence of agonist activity.

Table 4
Receptor-Binding Affinities^a and Antagonist Activities^b in Human PMNs

Compound	Receptor Affinity ^a IC ₅₀ (μM)	Antagonist Potency ^b IC ₅₀ (μM)	Agonist Activity ^c
7 MeFKP (dCha)Wr	1.8 (15)	0.085 (9)	No
8 MeFKP (dCha)Wr-CONH ₂	14 (5)	0.5 (3)	No
9 MeFKP (dCha)WR	11 (5)	0.7 (3)	No
10 MeFKPLWR	144 (1)	>1000 (3)	nd
11 Ac-F-[KP(dCha)Wr]	3.2 (40)	0.090 (5)	No
12 Ac-F-[OP(dCha)Wr]	0.28 (6)	0.012 (4)	No
4 YSFKPMPLaR	6.0 ^d	-	Yes
1 C5a65-74, ISHKDMLGR	>1000 ^e	-	-
C5a	0.0008 (9)	-	Yes

5 Number of experiments in parenthesis. Corrected for amino acid content.

Square brackets indicate cyclic portion.

nd = not determined

^a 50% reduction in binding of ¹²⁵I-C5a to intact human PMNs

^b 50% reduction in myeloperoxidase secretion from human PMNs mediated by 100 nM C5a

^c Agonist activity in dose range 0.1 nM-1 mM

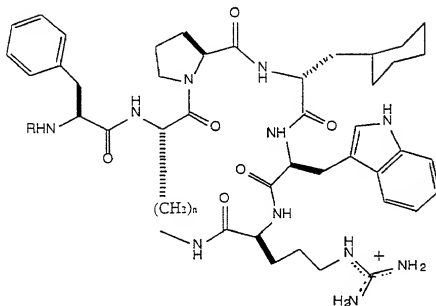
^d Finch et al, 1997; ^e Kawai et al, 1991

- 38 -

It has previously been proposed that the C-terminus of C5a and of agonist peptides is essential for activity, due to its interaction with a positively-charged Arg206 of the receptor (DeMartino et al, 1995). We confirm here that the C-terminal carboxylate is indeed important for activity (8 vs. 7), but wondered whether the origin of this effect might be due to hydrogen bonding between the carboxylate anion and the positively charged amine side chain of Lys. Conversion to the amide (8) certainly reduces both receptor-affinity and antagonist activity approximately 5-fold. Changing chirality of the Arg-C α (9 vs. 7) causes a similar reduction in activity, and replacing dCha with the less bulky Leu residue (10) is also detrimental to receptor binding. However, potency is recovered for cyclic compounds 11 and 12, in which an amide bond is tolerated at the C-terminus, consistent with the structural interpretation above that the advantage of the carboxylate in 7 may be associated with intramolecular hydrogen bonding. The replacement of this hydrogen bond in 7 with a covalent amide bond in 11 and 12 more effectively stabilizes the turn conformation.

Figure 5C compares C5aR binding and antagonist potency *in vitro* on human PMNs for compounds 15 and 17 with those for compound 7. Both 15 and 17 are potent inhibitors at nM concentrations of the action of C5a and the binding of 125 I-C5a to its receptor (e.g. 4, $K_b = 1.4$ nM). Their cyclic nature and the acetylation at the N-terminal phenylalanine both protect against the proteolytic degradation typically encountered by peptides, making such cyclic compounds more suitable than acyclic peptides as drug candidates. The results are shown in Table 5.

Table 5
 Receptor Binding and Antagonist Activity
 of Cyclic Molecules



5

Compound	n	R	Isomer [*]	Receptor Affinity μM	Agonist Activity
13	1	H	S-	9	No
14			R-	34	No
15	2	H	S-	0.3	No
16			R-	3.7	No
17	3	Ac	S-	0.3	No
11		Ac	R-	38	No
18	4	Ac	S-	3.2	No
12		Ac	R-	51	No

Refers to stereochemistry of Arg side chain.

- 40 -

Example 4 Cyclic Antagonists of C5a

Some examples of these cyclic antagonists and their apparent receptor-binding affinities and antagonist potencies are given in Tables 4, 5 and 6 as well as in
5 Figures 5 and 6. In the tables the single letter code for amino acids is used.

Fig.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204

Table 6
Effect of Cyclisation on Antagonist Binding Affinity and Antagonist Potency

PEPTIDE	pD ₂ ± SE ^a	IC ₅₀ (μM) ^a	(n)	pD ₂ ± SE ^b	IC ₅₀ (μM) ^b	(n)
11 AcF-[KPdChawR]	5.49 ± 0.22	3.2	4	7.07 ± 0.29	0.09	5
18 AcF-[OPdChawR]	6.44 ± 0.14*	0.4	9	7.30 ± 0.09	0.05	9
19 [FWPdChawR]	4.37 ± 0.36*	43	3	nd		
20 AcF-[KMdChawR]	4.81 ± 0.06	15	2	nd		
21 AcF-[KKdChawR]	3.94 ± 0.4	116	3	4.88	13	1
Effect of length of linker in cycle on antagonist binding affinity and antagonist potency						
22 AcF-[XPdChawR]	5.02 ± 0.07	9.5	3	4.71 ± 0.23	20	3
23 AcF-[X ² PdChawR]	4.77 ± 0.14*	17	3	6.09 ± 0.08*	0.8	4
11 AcF-[OPdChawR]	4.60 ± 0.06*	16	4	6.42 ± 0.10	0.4	4
24 AcF-[OPdChawR]	4.96 ± 0.03	11	3	6.73	0.2	1

H:\PCT\Kha.Masp\Amenda\PCT-00490_V02.doc 24/08/99

Table 6 (cont.)

PEPTIDE	pD2 ± Se ^a	IC50 (μ M) ^a	(n)	pD2 ± SE ^b	IC50 (μ M) ^b	(n)
14 F-[XpDChaWR]	4.39 ± 0.10*	41	3	nd		
16 F-[X ² pDChaWR]	5.42 ± 0.05	3.8	3	6.70 ± 0.04	0.4	3
25 F-[OPdChaWR]	5.51 ± 0.07	3.1	3	5.79 ± 0.34*	1.6	3
26 F-[KPdChaWR]	5.09 ± 0.08	8.1	3	5.55 ± 0.57*	2.8	3
Effect of L-Arg on antagonist binding affinity and antagonist potency						
17 AcF-[OPdChaWR]	6.57 ± 0.05*	0.3	3	7.91 ± 0.17*	0.01	3
13 F-[XpDChaWR]	4.98 ± 0.05	10	3	5.63 ± 0.13*	2.4	3
15 F-[X ² pDChaWR]	6.50 ± 0.04*	0.3	5	7.36 ± 0.13	0.04	3
27 F-[OPdChaWR]	7.21 ± 0.01*	0.06	3	7.41 ± 0.14	0.04	3
28 F-[KPdChaWR]	6.50 ± 0.12*	0.3	4	6.69 ± 0.04	0.2	3

- 43 -

- a pD_2/IC_{50} ; concentration of peptide resulting in 50% inhibition in the binding of [^{125}I]C5a to intact PMNs. The IC_{50} is the antilog of the mean pD_2 value
- b pD_2/IC_{50} ; concentration of peptide resulting in 50% inhibition in the ability of C5a (100 nM) to cause the release of MPO from PMNs

X = $(CH_2)_n-NH_2$

X² = $(CH_2)_2-NH_2$

pD_2 values are expressed as mean \pm SE

- 10 n represents the number of experiments performed

* Significant change in affinity/potency compared to NMeFKPdChaWR ($p < 0.05$)

indicates isomer number

- 15 These results demonstrate:

(1) that the cyclic molecules have higher apparent receptor affinity and may be more potent antagonists than acyclic (linear) peptides,

- (2) that one of the two possible cyclic diastereomers is consistently favoured for binding to the C5a receptor, and it is surprisingly the opposite stereochemistry (L-arginine) to that favoured in the linear compounds (D-arginine)
- 20

- (3) that the cycles have an optimum ring size for receptor-binding,
- 25

(4) that there is a pseudo-linear relationship between log (antagonist potency) and log (receptor affinity).

- Tables 5 and 6 list the C5a receptor affinities of some examples of cyclic antagonists of C5a, and their ability to bind to, and inhibit, binding of C5a to human PMNs is illustrated in Figure 6. Surprisingly these data show that the L-arginine is preferred over the D-arginine, in contrast to the linear compound 7 in which the D-arginine confers higher affinity for the receptor than does L-arginine. The data also show that the size of the macrocycle is optimal when $n = 2$ or 3, the smaller cycle
- 30
- 35

- 44 -

where $n = 1$ and the larger cycle when $n = 4$ being clearly less active. This requirement for a tightly constrained cycle is probably due to the need to correctly position the attached side chain residues of, for example, Trp, dCha, Arg and Phe for interaction with the receptor.

Example 5 Computer Modelling of Antagonist Structures

Figure 7 compares the computer-modelled structure of the cyclic antagonist **12** with the NMR solution structure for the acyclic antagonist **7**. These backbone structures are strikingly similar, and strongly suggest that the receptor-binding conformations of these molecules involve the same turn structure. Compound **12**, a more potent antagonist than **11**, also has a shorter linker, which tightens the turn and slightly alters the conformational space accessible to the key side chains of Phe, dCha, Trp and Arg. The conformational limitations placed on the hexapeptide derivative **12** by the cycle are responsible for a $\geq 10^4$ increase in receptor-binding affinity over the conformationally flexible decapeptide C-terminus of C5a (**1**, Table 2).

There is a correlation between binding affinities and antagonist potency for the site 2 antagonists (compounds **7-12**, Table 2). It thus appears that antagonist potency is dependent upon changes that occur at site 2 alone. Without wishing to be bound by any proposed mechanism, we believe that this may be because the mechanism of antagonism is related to conformational change to a turn conformation induced by **7** at site 2 of the receptor.

Example 6 Characterisation of C5aRs on Different Cells

Currently there is no information about different types of C5aRs. We have previously shown marked differences in the responsiveness of different cells containing functional C5aRs to agonists (Sanderson et al, 1994, 1995; Finch et al, 1997) and we can now provide more

- 45 -

information by examining potency and efficacy of selective agonists and antagonists relative to human recombinant C5a. For agonists, the tissue or cell selectivity may reveal functionally different receptors. Binding assays using
5 human PMNs, U937 cells, or circulating monocytes are used to determine affinities for C5aRs. Selectivity for different C5aRs is ascertained by differential antagonism. This combined approach allows pharmacological
10 characterisation of new agonists or antagonists, and may lead to a potential functional classification of C5aRson different cells.

Example 7(a) Neutropenia and C5a Antagonism In Vivo

Compounds were evaluated in an acute model of
15 C5a-induced neutropenia. Transient neutropenia maximises 5 min after i.v. C5a and is profound, with >90% of circulating neutrophils disappearing from circulation at effective doses of C5a, as shown in Figure 8. The
20 neutropenia is due to transient adherence of circulating neutrophils to the vascular endothelium. Preliminary data show that neutropenia caused by i.v. C5a is blocked by a C5a antagonist. For example, F-[OPdChaWR], (1 mg/kg), given prior to 2µg C5a i.v., inhibits C5a-induced
25 neutropenia *in vivo* (Figure 8).

Example 7(b) Inhibition of Lipopolysaccharide-induced Effects by C5a Antagonists

LPS causes rapid neutropenia in rats. If this
30 effect of LPS is blocked by C5a antagonists, then C5a may be of major importance in the acute effects of LPS, and the results shown in Figure 9 were in agreement with this hypothesis. C5a antagonists were injected (bolus i.v.) 10 min prior to challenge with LPS. Rats were anaesthetised, and blood samples (0.3ml) were taken for measurements of
35 PMNs. PMNs are isolated and quantified. Preliminary results show that F-[OPdChaWR], (1 mg/kg), given prior to i.v.LPS, inhibits neutropenia..

- 46 -

The results also indicate that the C5a antagonist inhibits the increase in haematocrit caused by LPS, showing that vascular leakage of serum caused by LPS is also inhibited.

5 These results demonstrate that C5a receptor antagonists, such as those described in this invention, may have therapeutic utility in septicemic individuals. The ability to inhibit the adherence of PMNs to vascular endothelium, and to inhibit the vascular leakage to LPS as
10 shown by the reduction of haematocrit values, indicates powerful anti-inflammatory effects of these compounds against proinflammatory stimuli activating the complement system, such as endotoxin or LPS.

15 Example 8 In Vivo Activity of Cyclic C5a Antagonists

Preliminary experiments in rats have revealed that the cyclic antagonists summarized in Table 5 are active at less than 20 mg/kg as anti-inflammatory agents in suppressing the onset of either carrageenan-induced paw
20 oedema or adjuvant-induced polyarthritis. The maximally effective dosages for even moderately-effective antagonists are 10 mg/kg or less, given i.p. or p.o. Many anti-inflammatory drugs currently used in humans were initially evaluated in such assays, and also showed activity in these
25 rat models of inflammation. These preliminary indications of efficacy *in vivo* indicate that C5a antagonists have therapeutic potential in human inflammatory conditions.

Using the rat carrageenan paw oedema assay, we found that a compound, AcF-[O-P-dCha-W-r], which is
30 100 times less active than **17** *in vitro* as a C5a antagonist in PMNs, has some *in vivo* activity in rats given 1 mg/kg of the compound I.P. 30 min prior to the carrageenan injection. Paw swelling was measured for up to 4.5 hr. The results, shown in Figure 10, suggest that even this weak C5a
35 antagonist significantly inhibits development of the oedema after 180 and 270 min. This anti-inflammatory activity suggests that C5a receptor antagonists, such as those

- 47 -

described in this invention, may have therapeutic activity in diseases involving vascular leakage following inflammatory stimuli.

In recent years there have been many attempts to mimic β - and γ - turn peptides that represent bioactive protein surfaces, resulting in notable mimetics for RGD (arginine-glycine-aspartate) peptides, somatostatin and opioid peptides, to name a few derived through structure-activity relationships (see for example Marraud and Aubry, 1996; Fairlie et al, 1995). Most of these examples preserve a turn structure through cyclisation of the peptide. On the other hand, there are comparatively few short acyclic peptides that have been found to have substantial turn structure in solution (Dyson et al, 1988; Rizo and Gierasch, 1992; Pràcheur et al, 1994). It is usually argued that short acyclic peptides adopt a myriad of solution structures that may include small populations of turn structures that are responsible for bioactivity.

This invention describes a series of conformationally-constrained turn-containing molecules that are preorganized for binding to the same G protein-coupled receptor(s) of human cells that are targeted by human C5a. The invention is applicable to other G protein-coupled receptors.

The principal feature of the compounds of the invention is the preorganized arrangement, which brings at least three hydrophobic groups and a charged group into neighbouring space, creating a hydrophobic surface 'patch'. These results enable the design and development of even more potent conformationally-constrained, small molecule antagonists of C5a.

In the light of the aforementioned prior art, it was surprising to find that a C-terminal carboxylate was not necessary in our compounds in order to obtain good receptor-binding or antagonist activity. The cyclic antagonists have an amide bond at the 'C-terminal' arginine position. The replacement of the carboxylate in 7 with a

- 48 -

covalent amide bond effectively stabilises the required turn conformation.

Cyclic and non-peptidic antagonists have several important advantages over peptides as drugs. The cycles described in this invention are stable to proteolytic degradation for at least several hours at 37°C in human blood or plasma, or in human or rat gastric juices or in the presence of digestive enzymes such as pepsin, trypsin and chymotrypsin. In contrast, short peptides composed of L-amino acids are rapidly degraded to their component amino acids within a few minutes under these conditions. A second advantage lies in the constrained single conformations adopted by the cyclic and non-peptidic molecules, whereas acyclic or linear peptides are flexible enough to adopt several structures in solution other than the required receptor-binding structure. Thirdly, cyclic and non-peptidic compounds such as those described in this invention are usually more lipid-soluble and more pharmacologically bioavailable as drugs than peptides, which can rarely be administered orally. Fourthly, the plasma half-lives of cyclic and non-peptidic molecules are usually longer than those of peptides.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

References cited herein are listed on the following pages, and are incorporated herein by this reference.

- 49 -

REFERENCES

- Abbenante, G., Fairlie, D.P., Gahan, L.R., Hanson, G.R.,
Pierens, G.K. and van den Brenk, A.L.
5 J. Am. Chem. Soc., 1996 118 10384-10388
- Bandekar, J.
Vib. Spectros., 1993 5 143-173
- 10 Bean, J.W., Peishoff, C.E. and Kopple, K.D.
Int. J. Protein Res., 1994 44 223
- Cheng, X. M., Doherty, A. M., Nikam, S. S.
Curr. Med. Chem. 1994 1 271-312
- 15 Coles, M., Sowemimo, V., Scanlon, D., Munro, S.L.A.,
Craik, D.J.
J. Med. Chem., 1993 36 2658
- 20 DeMartino, J.A., Konteatis, Z.D., Siciliano, S.J.,
Van Riper, G., Underwood, D.J., Fischer, P.A.,
Springer, M.S.
J. Biol. Chem., 1995 270 15966-15969
- 25 DeMartino, J.A., Van Riper, G., Siciliano, S.J.,
Molineaux, C.J., Konteatis, Z.D., Rosen, H. Springer, M.S.
J. Biol. Chem., 1994 269 14446-14450.
- Drapeau, G., Brochu, S., Godin, D., Levesque, L., Rioux, F.
30 and Marceau, F.
Biochem. Pharm., 1993 45 1289- 1299
- Dyson, H.J., Rance, M., Houghten, R.A., Lerner, R.A. and
Wright, P.E.
35 J. Mol. Biol., 1988 201 161-200

- 50 -

Ember, J.A., Sanderson, S.D., Taylor, S.M., Kawahara, M.
and Hugli, T.E.

J. Immunol., 1992 148 3165-3173

- 5 Fairlie, D.P., Abbenante, G. and March, D.
Curr. Med. Chem., 1995 2 672-705

Fairlie, D.P., Whitehouse, M. and Broomhead, J.
Chem. Biol. Interact., 1987 61 277-291

10

Finch, A.M., Vogen, S.M., Sherman, S.A., Kirnarsky, L.,
Taylor, S.M., and Sanderson, S.D.J. Med Chem., 1997 40 877

- 15 Gerard, N and Gerard, C.
Nature, 1991 349 614-617

Gerard, C and Gerard, N.P.
Ann. Rev. Immunol., 1994 12 775-808

- 20 Haviland, D.L., McCoy, R.L., Whitehead, W.T., Akama, H.,
Molmenti, E.P., Brown, A., Haviland, J.C., Parks, W.C.,
Perlmutter, D.H. and Wetsel, R.A.
J. Immunol., 1995 154 1861-1869

- 25 Hutchinson, E.G. and Thornton, J.M.
Protein Sci., 1994 3 2207-2216

Ihara, M., Fukuroda, T., Saeki, T., Nishikibe, M., Kojiri,
K., Suda, H. and Yano, M.

- 30 Biochem. Biophys. Res. Comm., 1991 178 132-137

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T.,
Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K.,
Nishikibe, M., and Yano, M.

- 35 Life Sciences, 1992 50 247

- 51 -

- Kawai, M., Quincy, D.A., Lane, B., Mollison, K.W.,
Luly, J.R., Carter, G.W.
J. Med. Chem., 1991 34 2068-71
- 5 Kawai, M., Quincy, D.A., Lane, B., Mollison, K.W.,
Or, Y.-S., Luly, J.R., and Carter, G.W.
J. Med. Chem., 1992 35 220-223
- 10 Kessler, H., Diefenbach, B., Finsinger, D., Geyer, A.,
Gurrath, M., Goodman, S.L., Hoelzemann, G., Haubner, R.,
Jonczyk, A. et al
Lett. Pept. Sci., 1995 2 155-160
- Kohl, J., Lubbers, B., Klos, A., et al.
15 Eur. J. Immunol., 1993 23 646-652
- Konteatis, Z.D., Siciliano, S.J., Van Riper, G.,
Molineaux, C.J., Pandya, S., Fischer, P., Rosen, H.,
Mumford, R.A., and Springer, M.S.
20 J. Immunol., 1994 153 4200- 4204
- Marraud, M. and Aubry, A.
Biopolymers, 1996 40 45-83
- 25 Morgan, E.L., Sanderson, S.D., Scholz, W., Noonan, D.J.,
Weigle, W.O. and Hugli, T.E.
J. Immunol., 1992 48 3937-3942
- Pràcheur, Bossus, M., Gras-Masse, H., Quiniou, E.,
30 Tartar, A. and Craescu, C.T.
J. Biochem., 1994 220 415-425
- Rizo, J. and Gierasch, L.M.
Ann. Rev. Biochem., 1992 61 387
- 35 Sanderson, S.D., Ember, J.A., Kirnarsky, L., Sherman, S.A.,
Finch, A.M., Taylor, S.M.

- 52 -

- J. Med. Chem., 1994 37 3171-3180
- Sanderson, S.D., Kirnarsky, L., Sherman, S.A., Vogen, S.M.,
Prakesh, O., Ember, J.A., Finch, A.M. and Taylor, S.M.
- 5 J. Med. Chem., 1995 38 3669-3675
- Siciliano, S.J., Rollins, T.E., DeMartino, J.,
Konteatis, Z., Malkowitz, L., VanRiper, G., Bondy, S.,
Rosen, H. and Springer, M.S.
- 10 Proc. Nat. Acad. Sci. USA, 1994 91 1214-1218.
- Sim, E.
The Natural Immune System. Humoral Factors., 1993,
IRL Press, Oxford University Press, Oxford.
- 15 Tempero, R.M., Hollingsworth, M.A., Burdick, M.D.,
Finch, A.M., Taylor S.M., Vogen, S.M., Morgan, E.L., and
Sanderson, S.D.
- J. Immunol., 1997 158 1377-1382
- 20 Stradley, S., Rizo, J., Bruch, M., Stroup, A. and
Gierasch, L.
Biopolymers, 1990 22 263-287.
- 25 Walker W.R. and Whitehouse, M.W.
Agents & Actions, 1978 8 85
- Ward, J.R. and Cloud, R.S.
J. Pharmacol. Exp. Ther., 1966 152 116
- 30 Whaley, K.
Complement in Health and Disease. Immunology and Medicine
Series, Ed. Reeves, W. G., 1987, MTP Press Ltd, Lancaster
- 35 Whitehouse, M.W.
Handbook of Animal Models for the Rheumatic Diseases, Eds.
Greenwald, R.A., Diamond, H.S., Vol. 1, pp 3-16 CRC Press

- 53 -

Winter, C.A. and Nuss, G.W.

Arth. & Rheumatism, 1966 9 394

- 5 Zhang, X., Boyar, W., Galakatos, N. and Gonella, N.C.
Protein Sci., 1997 6 65-72

Zuiderweg, E.R.P., Nettesheim, D.G., Molison, K.W., Carter,
G.W.

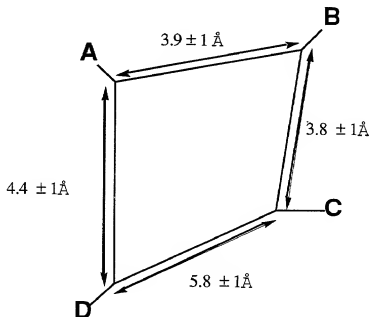
- 10 Biochemistry, 1989 28 172-185; 29 2895-2905.

- 54 -

CLAIMS:

1. A compound which is an antagonist of a G protein-coupled receptor, which has no agonist activity, and which has a cyclic or constrained acyclic structure adapted to provide a framework of approximate dimensions as set out in Structure I:

Structure I



10

where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives, and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

15 where the critical amino acid side chains are designated by A, B, C and D, where

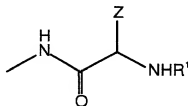
A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position;

20 B is any common or uncommon, aromatic amino acid side chain which serves to position an aromatic side-chain in this position;

- 55 -

C is any common or uncommon, hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position;

- 5 D is any common or uncommon, aromatic amino acid which serves to position an aromatic side-chain in this position, and has the structure:



10

where Z is indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or a derivative thereof; and R¹ is H or an alkyl, aromatic, acyl or aromatic-
15 acyl group.

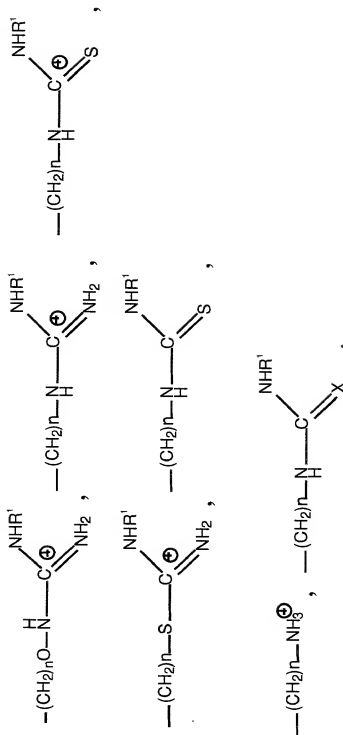
15

2. An antagonist according to Claim 1, in which the G protein-coupled receptor is the C5a receptor.

3. An antagonist according to Claim 1 or Claim 2, in which

20

A is one of the following side-chains



H:\PCL\arx\keep\specna\PCT-00490_VS.doc 24/08/99

- 57 -

or another mimetic of an arginine side chain;
where

X is NCN, NNO_2 , CHNO_2 or NSO_2NH_2 ;

n is an integer from 1 to 4, and

- 5 R^1 is H or an alkyl, aryl, CN, NH_2 , OH, $-\text{CO}-\text{CH}_2\text{CH}_3$,
 $-\text{CO}-\text{CH}_3$, $-\text{CO}-\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{CO}-\text{CH}_2\text{Ph}$, or $-\text{CO}-\text{Ph}$;

B is an indole, indole methyl, benzyl, phenyl, naphthyl,
naphthyl methyl, cinnamyl group, or any other derivative of
10 the aromatic group; and

C is D- or L-cyclohexylalanine (Cha), leucine,
valine, isoleucine, phenylalanine, tryptophan or
methionine.

4. An antagonist according to Claim 3, in which
15 R^1 is methyl, ethyl, propyl, or butyl.

5. An antagonist according to any one of Claims 1 to
4, which is a constrained acyclic compound, and comprises a
type II β -turn.

6. An antagonist according to Claim 5, in which the
20 type II β -turn comprises a γ -turn within the type II β -
turn.

7. An antagonist according to any one of claims 1 to
4, which is a cyclic peptide or peptide derivative.

8. An antagonist according to any one of Claims 1 to
25 4, of formula

$\text{Ac-phe-[lys-pro-(dCha)-trp-arg]}$ or

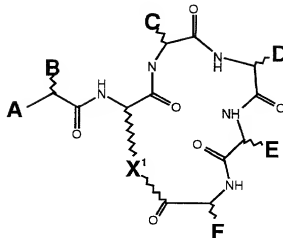
$\text{Ac-phe-[orn-pro-(dCha)-trp-arg]}$

9. An antagonist according to any one of Claims 1 to
7 in which A is L-arginine.

- 30 10. An antagonist according to Claim 1, which has
antagonist activity against C5aR , has no agonist activity
against C5a , and has the general formula:

- 58 -

Structure II



where A is H, alkyl, aryl, NH₂, NHalkyl, N(alkyl)₂,
5 NHaryl or NHacyl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or
indole group, or the side chain of a D- or L-amino acid
selected from the group consisting of phenylalanine,
homophenylalanine, tryptophan, homotryptophan, tyrosine,
10 and homotyrosine;

C is the side chain of a D-, L- or homo-amino
acid selected from the group consisting of proline,
alanine, leucine, valine, isoleucine, arginine, histidine,
aspartate, glutamate, glutamine, asparagine, lysine,
15 tyrosine, phenylalanine, cyclohexylalanine, norleucine,
tryptophan, cysteine and methionine;

D is the side chain of a D- or L-amino acid
selected from the group consisting of cyclohexylalanine,
homocyclohexylalanine, leucine, norleucine, homoleucine,
20 homonorleucine and tryptophan;

E is the side chain of a D- or L-amino acid
selected from the group consisting of tryptophan and
homotryptophan;

F is the side chain of a D- or L-amino acid
25 selected from the group consisting of arginine,
homoarginine, lysine and homolysine; and

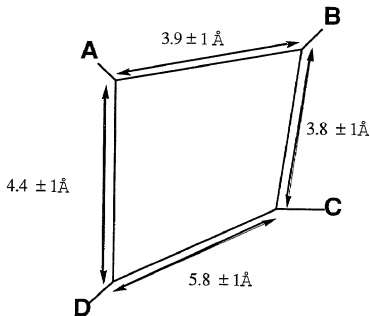
- 59 -

X^1 is $-(CH_2)_nNH-$ or $(CH_2)_nS-$, $-(CH_2)_2O-$, $-(CH_2)_3O-$, $-(CH_2)_3-$, $-(CH_2)_4-$, or $-CH_2COCHRNH-$, where R is the side chain of any common or uncommon amino acid, and

where n is an integer of from 1 to 4,

- 5 11. An antagonist according to Claim 10, in which F is a L-amino acid.
12. An antagonist according to Claim 11, in which F is L-arginine.
13. An antagonist according to any one of Claims 10 to 12, selected from the group consisting of compounds **11**, **12**, **13**,
10 **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **27** and **28**.
14. An antagonist according to any one of Claims 3 and 10 to 13, in which n is 2 or 3.
- 15 15. A compound which is an agonist of a G protein-coupled receptor, and which has structure III

Structure III



20 where the numerals refer to distances between C_α carbons of amino acids or their analogues or derivatives,

- 60 -

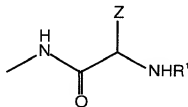
and A, B, C and D are not necessarily on adjacent amino acids, or analogues or derivatives thereof; and

where B is a non-aromatic amino acid, and

A is any common or uncommon, basic, charged amino acid side chain which serves to position a positively charged group in this position;

C is any common or uncommon, hydrophobic amino acid side chain which serves to position any alkyl, aromatic or other group in this position; and

D is any common or uncommon, aromatic amino acid which serve to position an aromatic side-chain in this position, and has the structure:



where Z is indole, indole methyl, benzyl, benzene, naphthyl, naphthyl methyl, or a derivative thereof; and

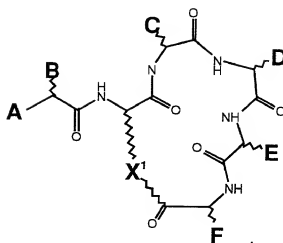
R is H or an alkyl, aromatic, acyl or aromatic-acyl group.

A compound according to Claim 15, where B is the D- or L-form of alanine, leucine, valine, norleucine, glutamic acid, aspartic acid, methionine, cysteine, isoleucine, serine or threonine.

A compound according to Claim 15 or Claim 16, in which the compound is of structure IV,

- 61 -

Structure IV



5

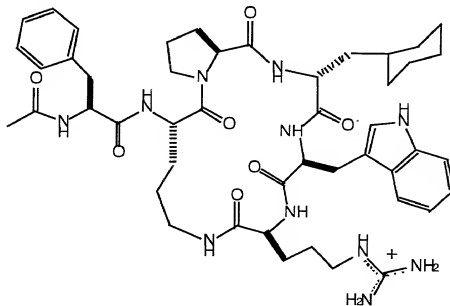
where E is any amino acid other than tryptophan and homotryptophan, and

F is the side chain of a D- or L-amino acid selected from the group consisting of arginine, homoarginine, lysine and homolysine.

10

18. A compound according to any one of Claims 15 to 17, wherein the compound is an agonist of C5a.

19. A compound according to Claim 10, of structure



- 62 -

20. A composition comprising a compound according to any one of Claims 1 to 19, together with a pharmaceutically-acceptable carrier or excipient.
21. A method of treatment of a pathological condition
5 mediated by a G protein-coupled receptor, comprising the step of administering an effective amount of a compound according to any one of Claims 1 to 19, to a mammal in need of such treatment.
22. A method according to Claim 21, wherein the
10 condition mediated by a G protein-coupled receptor involves overexpression or underregulation of C5a.
23. A method according to Claim 21, wherein the condition is selected from the group consisting of
15 rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, multiple sclerosis, lung injury and extracorporeal post-dialysis syndrome.
- 20 24. Use of a compound according to any one of Claims 1 to 19 in treatment of a pathological condition mediated by a G protein-coupled receptor.
25. Use according to Claim 24, in which the condition is mediated by C5a.
- 25 26. Use according to Claim 25, in which the condition mediated by G protein-coupled receptors involves overexpression or underregulation of C5a.
27. Use according to any one of Claims 24 to 26, in which the condition is selected from the group consisting
30 of rheumatoid arthritis, adult respiratory distress syndrome (ARDS), systemic lupus erythematosus, tissue graft rejection, ischaemic heart disease, reperfusion injury, septic shock, psoriasis, gingivitis, atherosclerosis, Alzheimer's disease, multiple sclerosis, lung injury and
35 extracorporeal post-dialysis syndrome.
28. Use of a compound according to any one of Claims 1 to 19 in the manufacture of a medicament for the

\\me1b01\home9\51neone\Keep\Spec\1\CT-99490.vs.doc

- 63 -

treatment of a condition mediated by a G protein-coupled receptor.

29. Use according to Claim 28, in which the condition is mediated by C5a.

5 30. Use according to Claim 29, in which the condition mediated by G protein-coupled receptors involves overexpression or underregulation of C5a.

31. A compound according to Claim 1, substantially as
10 hereinbefore described with reference to the examples and drawings.

32. A method according to Claim 21, substantially as hereinbefore defined with reference to the examples and drawings.

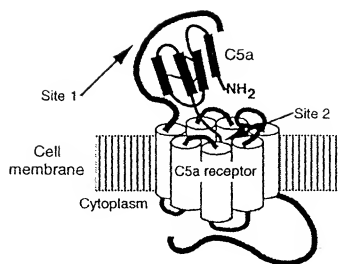


Fig. 1

2/12

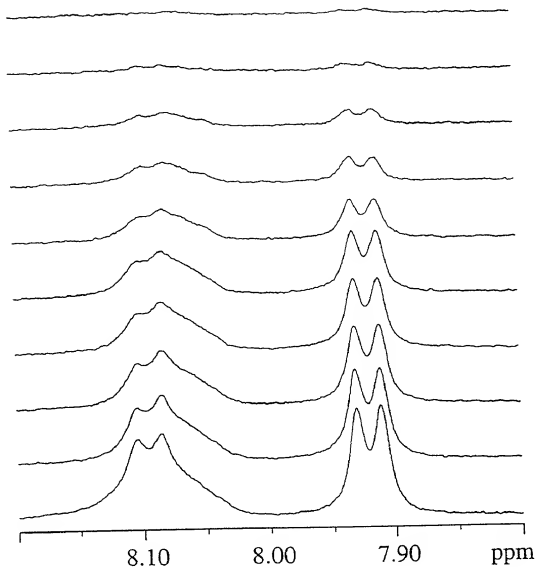


Fig. 2

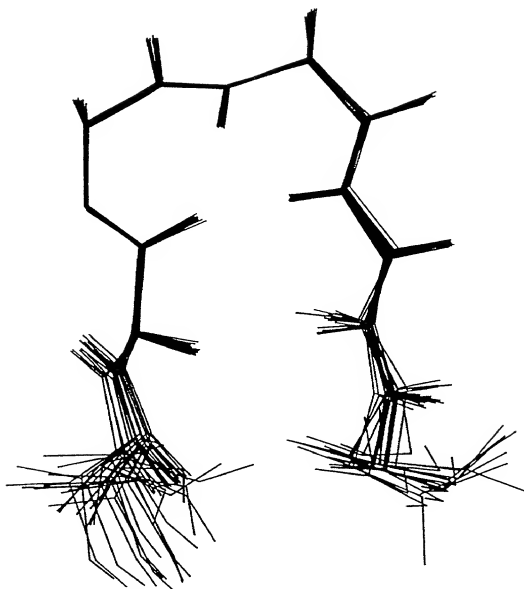


Fig. 3

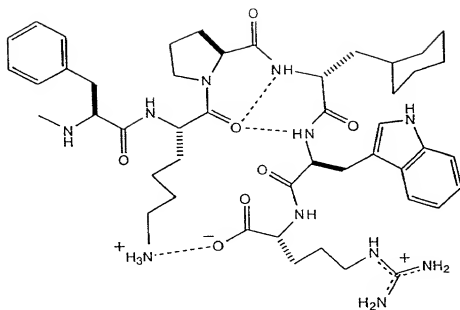


Fig. 4

5/12

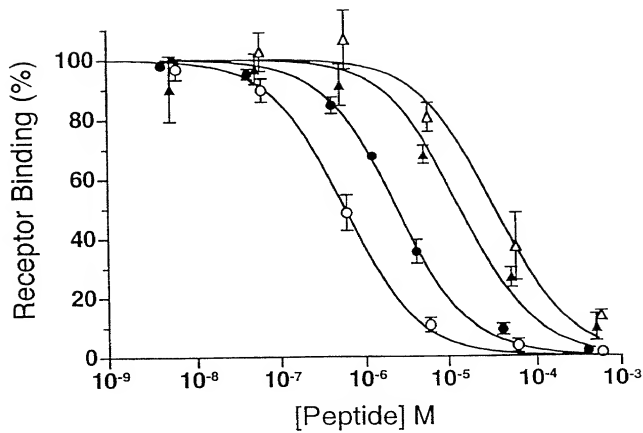


Fig. 5a

6/12

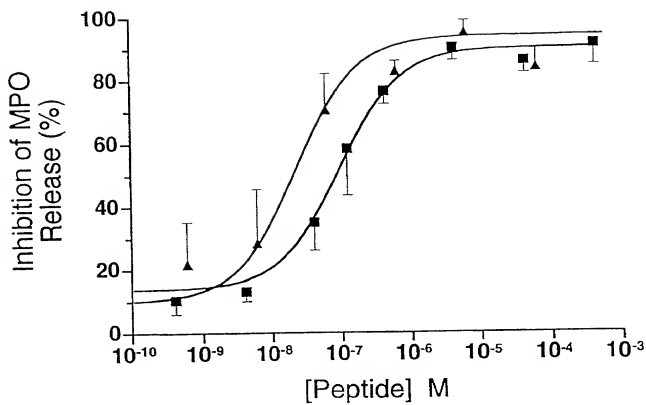
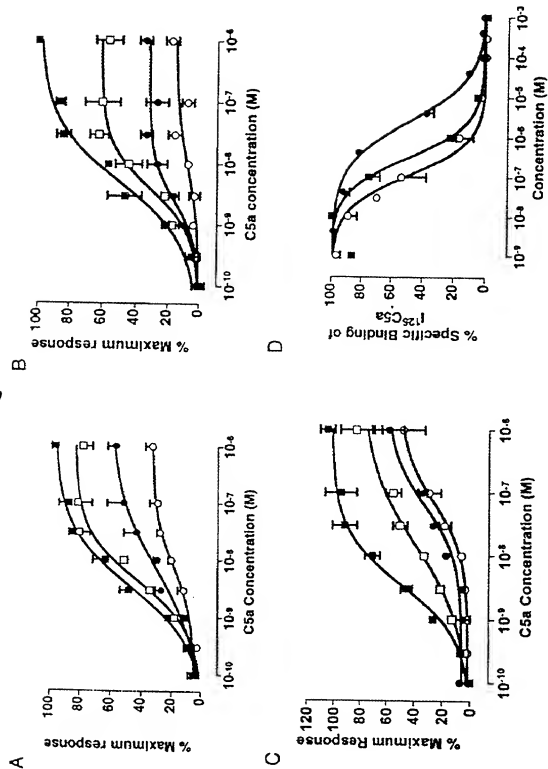


Fig. 5 b

7/12

Fig. 5c



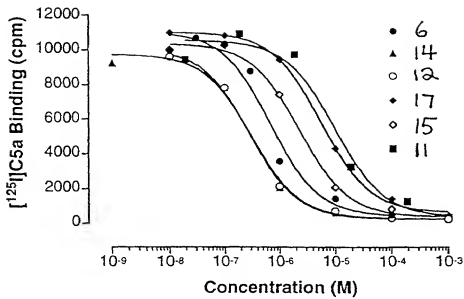
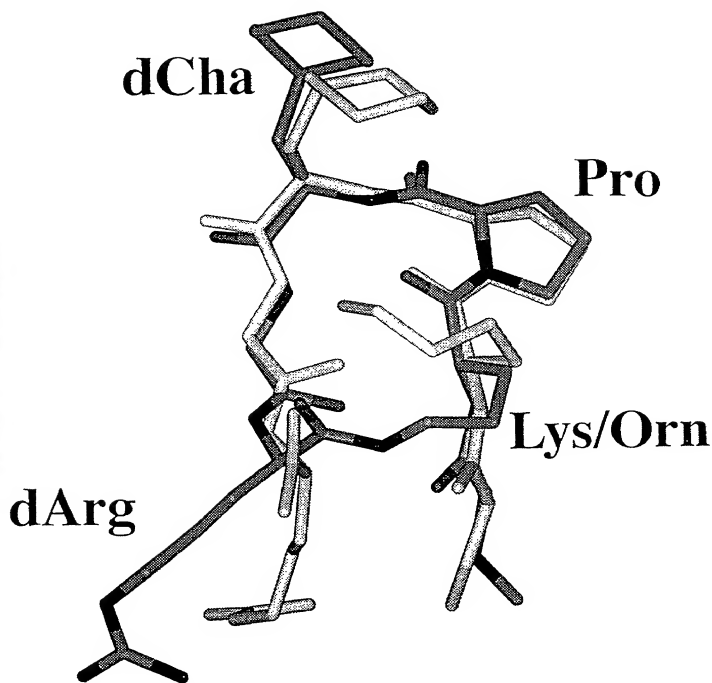


Fig. 6

*Fig. 7*

10/12

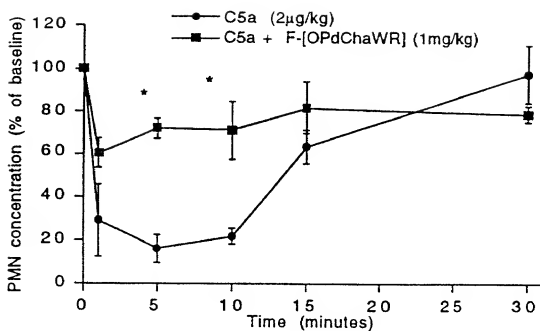


Fig. 8

11/12

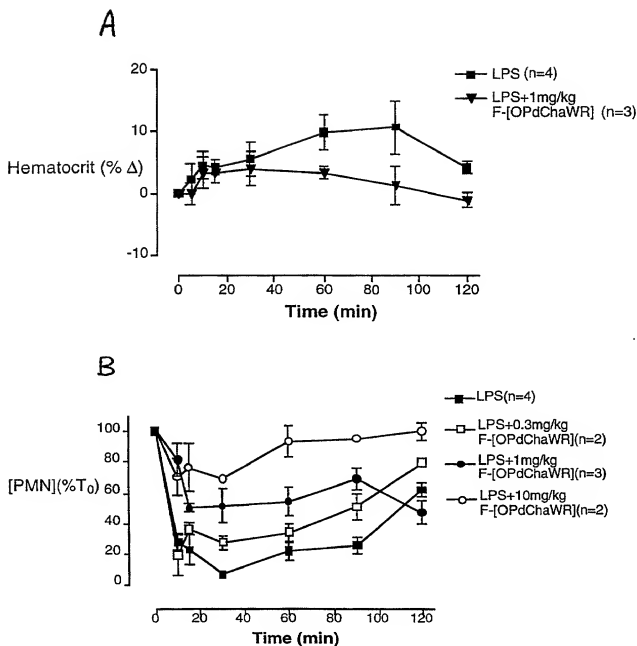
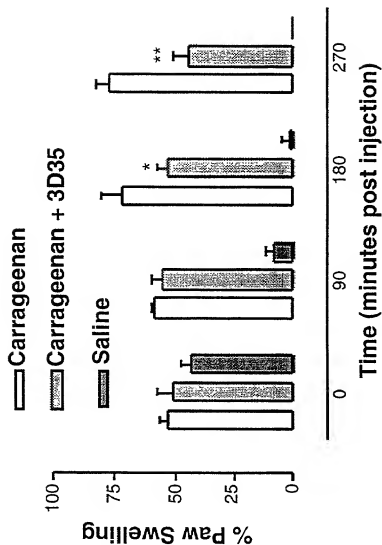


Fig. 9

12/12

*Fig. 10*

Declaration, Power Of Attorney and Petition

Page 1 of 3

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

CYCLIC AGONISTS AND ANTAGONISTS OF C5a RECEPTORS AND G PROTEIN-COUPLED RECEPTORS

the specification of which

☐ is attached hereto.

☒ was filed on December 23, 1999 as

Application Serial No. 09/446,109

and amended on _____.

☒ was filed as PCT international application

Number PCT/AU98/00490

on 25 June 1998,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
PO 7550	AUSTRALIA	25 June 1997	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)
PCT/AU98/00490	25 June 1998	

And we (I) hereby appoint: Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,529; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters, Reg. No. 28,870; Robert T. Pous, Reg. No. 29,999; Charles L. Gholz, Reg. No. 26,395; Vincent J. Sunderdick, Reg. No. 29,004; William E. Beaumont, Reg. No. 30,996; Robert F. Gnuse, Reg. No. 27,295; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 32,884; Robert W. Hahl, Reg. No. 33,893; Richard L. Treanor, Reg. No. 36,379; Steven P. Weihrouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Chinn, Reg. No. 34,305; Steven E. Lipman, Reg. No. 30,611; Carl E. Schlier, Reg. No. 34,426; James J. Kulbaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,299; J. Derek Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Christina M. Gadiano, Reg. No. 37,628; Jeffrey B. McIntyre, Reg. No. 36,867; Paul E. Rauch, Reg. No. 38,594; William T. Enos, Reg. No. 33,128; and Michael E. McCabe, Jr., Reg. No. 37,182; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

David FAIRLIE
NAME OF FIRST ~~SOLE~~ INVENTOR

Residence: 73 Trevallan Drive
Springwood, QLD 4127

AUSTRALIA

Citizen of: AUSTRALIA

Post Office Address: same as above

Signature of Inventor

Date

16th February, 2000

200
Stephen Maxwell TAYLOR
NAME OF SECOND JOINT INVENTOR

[Signature]
Signature of Inventor

19/2/2000
Date

Angela Monique FINCH
NAME OF THIRD JOINT INVENTOR

[Signature]
Signature of Inventor

20/3/2000
Date

Allan WONG
NAME OF FOURTH JOINT INVENTOR

[Signature]
Signature of Inventor

8 March, 2000
Date

NAME OF FIFTH JOINT INVENTOR

Signature of Inventor

Date

8 Perdita Street
Residence: Bellbird Park, QLD 4300

AUSTRALIA

Citizen of: AUSTRALIA

Post Office Address: same as above

Pioneer Drive, Narangba, QLD
Residence: 4502, AUSTRALIA

Citizen of: AUSTRALIA

Post Office Address: same as above

19 Gray Avenue
Residence: Bundaberg, QLD 4670, AUSTRALIA

Citizen of: AUSTRALIA

Post Office Address: same as above

Residence: _____

Citizen of: _____

Post Office Address: _____